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The regulation of myosin II in *Dictyostelium*

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Abstract

Dictyostelium conventional myosin (myosin II) is an abundant protein that plays a role in various cellular processes such as cytokinesis, cell protrusion and development. This review will focus on the signal transduction pathways that regulate myosin II during cell movement. Myosin II appears to have two modes of action in *Dictyostelium*: local stabilization of the cytoskeleton by myosin filament association to the actin meshwork (structural mode) and force generation by contraction of actin filaments (motor mode). Some processes, such as cell movement under restrictive environment, require only the structural mode of myosin. However, cytokinesis in suspension and uropod retraction depend on motor activity as well. Myosin II can self-assemble into bipolar filaments. The formation of these filaments is negatively regulated by heavy chain phosphorylation through the action of a set of novel alpha kinases and is relatively well understood. However, only recently it has become clear that the formation of bipolar filaments and their translocation to the cortex are separate events. Translocation depends on filamentous actin, and is regulated by a cGMP pathway and possibly also by the cAMP phosphodiesterase RegA and the p21-activated kinase PAKa. Myosin motor activity is regulated by phosphorylation of the regulatory light chain through myosin light chain kinase A. Unlike conventional light chain kinases, this enzyme is not regulated by calcium but is activated by cGMP-induced phosphorylation via an upstream kinase and subsequent autophosphorylation.

Keywords: *Dictyostelium*, myosin; Cortical tension; Signal transduction; cGMP

Introduction

The social amoebae *Dictyostelium discoideum* live as single cell in the soil where they feed on bacteria. Their prey is traced by chemotaxis towards secretion products of bacteria, such as folic acid and pterins. Upon food depletion, the cells enter a developmental cycle that is regulated by cAMP. The pulsatile secretion of this compound, that also serves as a chemoattractant, leads to the aggregation of the cells into clumps of about 100,000 cells. The aggregates subsequently develop into fruiting bodies that are composed of dead stalk cells and viable spores. When food becomes available again, the spores hatch into amoebae that are capable of mitotic reproduction.

Dictyostelium has been used widely as a model organism to study cell movement and chemotaxis. The basic motility apparatus is thought to be composed of 30 different actins, one conventional myosin (myosin II) and 11 unconventional myosins; no homologs of intermediate filament components could be detected in the completed genome (De la Roche and Cote, 2001; Loomis and Kuspa, 2005). The actin cytoskeleton is regulated by a vast amount of proteins, many of which have been studied in some detail (reviewed in Affolter and Weijer (2005) and Loomis and Kuspa (2005)).

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The functions of both conventional and unconventional myosins in *Dictyostelium* have also been investigated to some extent (reviewed in De la Roche and Cote (2001)). This short review will focus on the role of myosin II in cell movement with emphasis on the signal transduction pathways regulating myosin II.

Myosin structure and function

Myosin II structure

Dictyostelium contains a single myosin II complex that is composed of two myosin heavy chains (MHC), two essential light chains (ELC) and two regulatory light chains (RLC) (Fig. 1) (Chisholm et al., 1988; Tafuri et al., 1989; Warrick et al., 1986). Each heavy chain contains an N-terminal globular motor domain, a neck region with two IQ domains that bind the light chains, and a C-terminal tail region that forms a coiled coil with the second heavy chain molecule. The motor domain enables myosin to slide over actin filaments in the course of which ATP is hydrolyzed. The myosin II heterohexamer can spontaneously assemble into filaments composed of 10–20 myosin hexamers (Yumura and Kitanishi-Yumura, 1990). It is only these filaments that can perform the cellular myosin II functions, since mutant myosin molecules that are unable to form filaments, but retain ATPase activity, cannot rescue the myosin knockout phenotypes (Egelhoff et al., 1993; Liu et al., 1998).

Myosin has two modes of action

The phenotypes of the different myosin mutants are most easily understood if we assume two modes of action: one is local stabilization of the actin cytoskeleton by myosin filament association and the other is

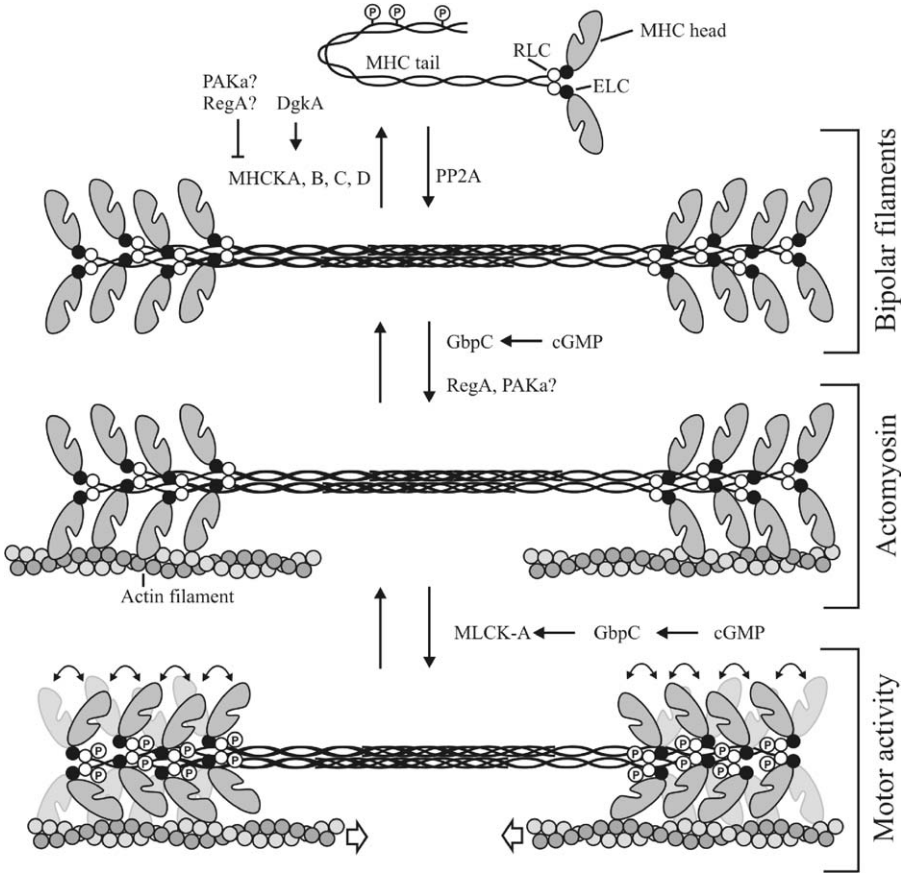

Fig. 1. Cartoon of myosin II action in *Dictyostelium*. Myosin II forms a hexamer composed of two myosin heavy chains (MHC) two regulatory light chains (RLC) and two essential light chains (ELC). Each heavy chain contains a head domain and a tail domain, which can be phosphorylated on three threonines by myosin heavy chain kinases (MHCKs). Spontaneous bipolar filament formation occurs upon dephosphorylation of the heavy chain by the phosphatase PP2A. Subsequently, the filaments can associate with filamentous actin, which is regulated by the cGMP-binding protein GbpC and possibly PAKa and RegA. The resulting actomyosin complex can exert a number of myosin II functions without requiring motor activity. The motor is activated considerably by phosphorylation of the regulatory light chain by myosin light chain kinase A (MLCK-A), which in turn is phosphorylated directly or indirectly by the cGMP-activated kinase GbpC.

ATP-powered contraction, for which the first mode is also required. Mutants that lack the gene encoding the heavy chain (*mhcA*$^-$ cells) display several defects. Firstly, cells are unable to divide when grown in shaking culture (Egelhoff et al., 1990). Although the mutants can undergo cytokinesis when grown on a solid support, they do so with decreased efficiency and use an abnormal "traction based" mechanism to finalize the process (De Lozanne and Spudich, 1987). These findings correspond to the observation that GFP-fused myosin is heavily enriched in the cleavage furrow of dividing cells, where it presumably contracts to pinch off the daughter cell. Secondly, development ceases shortly after the cells have aggregated (De Lozanne and Spudich, 1987). Thirdly, chemotaxis efficiency is diminished, which is caused by the dramatic reduction of cell polarity and the inability to suppress lateral pseudopodia (Heid et al., 2004; Wessels and Soll, 1990; Wessels et al., 1988). Cell movement is further hampered by a reduced speed of translocation, which may be caused by the inability to retract the uropod (Uchida et al., 2003; Wessels et al., 1988). Fourthly, when *mhcA*$^-$ cells are forced to chemotax under a layer of agarose, they are unable to proceed and will fragment (Laevsky and Knecht, 2003). This inabiltiy to push forward in a restrictive environment may also be the cause for exclusion of mutant cells from streams of wild-type amoebae (Shelden and Knecht, 1995; Xu et al., 2001). This defect may be caused by the reduced cell stiffness or cortical tension that these mutants display (Pasternak et al., 1989). Interruption of the gene encoding the RLC evokes the same aberrancies as disruption of the MHC-encoding gene does, with the exception of chemotaxis and movement in restrictive environments, on which there are no reports to date (Chen et al., 1994). Interestingly, mutants lacking the ELC display some but not all of the defects observed in *mhcA*$^-$ and *rlc*$^-$ mutants. These cells are not capable of growing in suspension, but development is less severely hampered: about 50% of the aggregates manage to produce viable spores (Chen et al., 1995). Moreover, chemotaxis efficiency is indistinguishable from wild-type cells, although the speed of the cells is reduced to 50%. Under agarose chemotaxis is also normal, as well as participation in cell streams (Xu et al., 2001; Laevsky and Knecht, 2003). Notably, myosin isolated from *elc*$^-$ cells does not display detectable actin-activated ATPase activity (Chen et al., 1995), nor do *elc*$^-$ mutants display any cytoskeletal contraction activity (Xu et al., 2001), indicating that the motor activity is lost in this mutant. These results strongly suggests that myosin that is devoid of motor activity is still capable of executing some of the myosin functions, such as chemotaxis and development. This seems to be contradicted by the earlier finding that MHC containing point mutations that nullify the motor activity cannot rescue the developmental phenotype of *mhcA*$^-$ mutants (Ruppel and Spudich, 1996). However, these myosin mutants also display altered actin binding capacity, since an in vitro actin sliding experiment led to the detachment of the mutant myosin filaments from the actin meshwork. This may also be the case for myosin from *rlc*$^-$ cells, that was reported to have very low motor activity in combination with aberrant filament aggregation (Chen et al., 1994). Thus, myosin II apparently exerts two actions in *Dictyostelium* (Fig. 1).

The first is the formation of filaments in the cytoskeleton which enhances cortical rigidity. The motor activity is not required for this function, provided that myosin filaments effectively bind to filamentous actin. Moreover, the specific localization of myosin filaments to the posterior and lateral regions of chemotaxing cells may prevent pseudopod formation at these positions, which would contribute to cell polarity and efficient chemotaxis. Apart from its role in cell polarity, this "structural" mode of myosin is also needed for movement in restrictive environment and multicellular development. Myosin presumably contributes to the cortical stability by cross-linking actin filaments as suggested by Xu et al. (2001). This is supported by the finding that actin filaments undergo structural changes upon myosin binding (Siddique et al., 2005). Furthermore, mutant myosins that can form filaments and retain ATPase activity, but are unable to bind actin filaments cannot rescue MHC knockout cells (Sasaki et al., 2000). However, it cannot be excluded that myosin filaments themselves function as structural components of the cortex as well.

The second mode of myosin action involves the ATP-driven translocation of actin filaments also termed motor activity. This activity is required for growth in suspension, maximal cell speed and fully normal development. Maximal cell speed is presumably achieved by active myosin-dependent retraction of the uropod (Uchida et al., 2003). This retraction is accompanied by a clear concentration of fluorescent signal in the back of myosin-GFP-expressing cells (Clow and McNally, 1999). Since binding of myosin filaments to F-actin is required for myosin to function as a molecular motor, this "motor" mode cannot function without the structural action of myosin II filaments.

The regulation of myosin II filament formation

As mentioned before, myosin cannot exert its function unless it forms bipolar filaments. It is therefore not surprising that filament formation is tightly regulated in *Dictyostelium*. Filament formation in vitro is known to be enhanced by acidic pH, magnesium ions, actin filaments and inhibited by MHC phosphorylation (Kuczmarski et al., 1987; Mahajan and Pardee, 1996;

Mahajan et al., 1989). It is unknown whether magnesium ions and protons regulate myosin filament formation in vivo. In mammalian cells it is known that intracellular magnesium levels can increase upon agonist stimulation, but it is as yet unknown whether this is also the case in *Dictyostelium* cells (Zhang and Melvin, 1992). Recently, it was shown that a *Dictyostelium* mutant lacking a Na^+–H^+ exchanger which has a defective pH homeostasis displays altered polarity during chemotaxis (Patel and Barber, 2005). Whether this defect is correlated with altered myosin filament formation remains to be determined. Dephosphorylation of the MHC is the most important mechanism that drives myosin II filament formation in *Dictyostelium.* MHC phosphorylation has been shown to occur at three threonines located in the coiled-coil tail (Luck-Vielmetter et al., 1990; Vaillancourt et al., 1988); upon phosphorylation of these threonines the coiled-coil destabilizes, causing disruption of the myosin II filaments (Liang et al., 1999). Studies in which these threonines were mutated to phosphate-mimicking aspartates have demonstrated that dephosphorylation is required for myosin filament formation and functioning (Egelhoff et al., 1993; Stites et al., 1998). The reciprocal experiment in which the threonines were mutated to non-phophorylatable alanines caused myosin overassembly in the cell cortex. Thus, phosphorylation of three threonines in the MHC tail inhibits filament formation.

MHC phosphorylation by myosin heavy chain kinases (MHCKs)

Myosin heavy chain is mainly phophorylated by three different MHCKs termed MHCK A through C, whereas a fourth homologous kinase MHCK D might also play a minor role (see for an overview Yumura et al., 2005). These kinases belong to the alpha-kinase protein family that is unrelated to canonical protein kinases. Apart from their catalytic domain, they possess a WD repeat domain that targets the MHCKs to their myosin II substrate (Steimle et al., 2001a). MHCK A – and most likely also MHCK B and C – phosphorylates the three previously mentioned threonines in the MHC tail that are critical for filament formation (Luck-Vielmetter et al., 1990; Vaillancourt et al., 1988). Furthermore, myosin mutants in which these threonines are mutated to alanines retained their resistence to filament disassembly upon MHCK A, B or C overexpression (Kolman et al., 1996; Rico and Egelhoff, 2003; Yumura et al., 2005). These findings strongly suggest that all MHCKs drive filament dissociation in the same way, namely phosphorylation of the three critical threonine residues in the MHC tail.

The differential localization of the MHCKs suggests that they may have separate functions in the cell. MHCK A is clearly enriched in anterior F-actin-rich regions, whereas MHCK B is mostly cytosolic with slight enrichment in the cleavage furrow of dividing cells (Steimle et al., 2001b; Yumura et al., 2005). Finally, MHCK C is clearly enriched in the cleavage furrow as well as in the posterior of cells that migrate under agarose (Liang et al., 2002; Yumura et al., 2005). Interestingly, MHCK A localization depends on the presence of filamentous actin, whereas MHCK C localization is myosin II dependent (Nagasaki et al., 2002; Steimle et al., 2001b). It has been suggested that MHCK A may function to disrupt myosin filaments at sites where new pseudopodia are being formed. MHCK C may function to liberate myosin hexamers from myosin aggregates that form in the cleavage furrow and in the posterior of the cell during uropod retraction, whereas MHCK B may serve a role in basal MHC phosphorylation levels (De la Roche et al., 2002a; Egelhoff et al., 2005; Nagasaki et al., 2002). Apart from this, all three MHCKs appear to have a role in cytokinesis as well (Yumura et al., 2005).

The mechanisms underlying activation and localization of MHCK A have been studied in more detail. Activation of MHCK A occurs primarily through autophosphorylation that is positively influenced by myosin II and acidic phospholipids, but is independent of cGMP, cAMP or calcium (Medley et al., 1990, 1992). More recently, it was shown that F-actin can also activate MHCK A, probably solely by increasing its autophosphorylation rate (Egelhoff et al., 2005). MHCK A binding to F-actin occurs through a novel coiled-coil domain (Steimle et al., 2002). Interestingly, it was recently discovered that this domain can also cross-link actin filaments, indicating that MHCK A may induce local protrusions not only by disrupting the myosin filaments, but also by directly influencing the actin cytoskeleton (Russ et al., 2006).

Diacylglycerol kinase (DGK)

A cDNA clone encoding a protein called MHC-PKC was previously reported to function as a MHC kinase (Ravid and Spudich, 1989). However, a recent report and the completed genome sequence have revealed serious errors in this cDNA (De La Roche et al., 2002b; Eichinger et al., 2005). Part of the cDNA is almost identical to a gene that encodes what appears to be the sole DGK in *Dictyostelium,* which was named DgkA. Other parts of the cDNA are homologous to a different part of the *Dictyostelium* genome or are of unknown origin. Like other canonical DGKs, DgkA phosphorylates the neutral lipid diacylglycerol to produce phosphatidic acid (De La Roche et al., 2002b; Ostroski et al., 2005). Furthermore, since a protein kinase domain is not present in DgkA, it seems unlikely

that DgkA directly phosphorylates MHC. Re-examination of targeted deletion mutants that were made with the original MHC-PKC cDNA has revealed that apparently the dgkA gene is disrupted in these mutants (Abu-Elneel et al., 1996; De La Roche et al., 2002b). The putative *dgkA* null (*dgkA*$^-$) mutants display several phenotypes that point to a link between lipid signaling and the MHCKs (Abu-Elneel et al., 1996). Firstly, membrane-associated kinase activity towards a *Dictyostelium* myosin tail fragment containing the three phosphorylatable threonines is strongly decreased in *dgkA*$^-$ cells. Moreover, MHC phosphorylation does not increase upon cAMP stimulation, although basal MHC phosphorylation levels were normal. Secondly, myosin II is overassembled in the cortex, as judged by immunolocalization of myosin and by the amount of myosin that resides in the Triton-insoluble fraction (the Triton-insoluble cell fraction is thought to reflect the cortical cytoskeleton). Thirdly, *dgkA*$^-$ cells display aberrant chemotaxis and cell polarity. These defects are quite similar to those associated with *mhckA* disruption (Kolman et al., 1996). In *mhckA* null mutants, 90% of Triton-insoluble MHC phosphorylation is lost and myosin II is overassembled in the Triton-insoluble fraction. The resemblance between these mutants suggests that DgkA may be required for MHCK A activity. Interestingly, a fusion protein between GFP and two of the C1 domains of DgkA localizes to the leading edge of chemotacting cells, similar to MHCK A (Rubin and Ravid, 2002; Steimle et al., 2001b, 2002). Further studies are required to elucidate the connection between DgkA and myosin II signaling.

Myosin heavy chain phosphatase

The proteins that dephosphorylate MHC are expected to play an important role in myosin filament assembly. It has been reported that the MHC phosphatase activity of lysates could not be stimulated by second messengers such as Ca^{2+} or cGMP nor did stimulation of cells with a chemoattractant result in a rapid increase in MHC phosphatase activity (Murphy and Egelhoff, 1999). These findings suggest that at least during chemotaxis, MHC phosphatase activity may not be regulated. The temporal and spatial regulation of the MHC phosphorylation state may be mainly executed by the MHCKs whereas the phosphatases continually dephosphorylate myosin hexamers in the cytosol. This is supported by the finding that the MHC phosphatase PP2A is located in the cytosol (Murphy et al., 1999). However, more detailed studies are required to elucidate the regulation of MHC dephosphorylation. Unfortunately, The only *Dictyostelium* MHC-specific phosphatase activity that has been identified to date is PP2A, on which unfortunately no further studies have been reported (Murphy and Egelhoff, 1999; Murphy et al., 1999).

The regulation of myosin filament association with F-actin

There is abundant evidence that dephosphorylation-driven myosin filament formation is a prerequisite for its cytoskeletal association and function. However, it has only recently become clear that a separate, independent mechanism drives these filaments to the cortex (Levi et al., 2002). When cells were treated with the F-actin-disturbing agent latrunculin A, a MHC–GFP fusion protein no longer translocated to the cortex, although filaments were still being formed in the cytosol. In the same study it was shown that a myosin II mutant lacking the head domain (which contains the actin-binding domain) also failed to move to the cortex, although cAMP-induced filament formation was unaffected. Remarkably, a GFP fusion with the myosin head domain that lacks the tail but still contains the light chain-binding domains was localized in the cytosol (L. Bosgraaf and P. v. Haastert, unpublished observations). This indicates that myosin filament formation is required for myosin association to the cytoskeleton. Thus, the translocation of myosin II to the cortex is composed of two steps: the formation of filaments in the cytosol and the F-actin-dependent association of the filaments with the cortex.

How are myosin filaments translocated to the cortex? One possibility is that myosin filaments by themselves simply bind to F-actin and are chased off at specific positions through the action of the MHCKs. Alternatively, myosin filaments could bind with different affinities to the various F-actin isoforms or conformations. In this respect, it is interesting to note that in non-muscle mammalian cells, α, β and γ actin sort to different regions of moving cells (Herman, 1993). It is as yet unknown whether the *Dictyostelium* actin isoforms also display this phenomenon and whether myosin filaments would prefer to bind to certain actin isoforms. Another noteworthy finding is that upon cofilin binding, actin filaments are changed in the twist of the filament (McGough et al., 1997). Such a conformational change could locally alter the affinity for myosin II filaments. A third possibility to regulate myosin filament translocation to the cortex is through the action of specific proteins. Interestingly, in mammalian cells, supervillin and Mts1 are capable of binding both myosin II and actin (Chen et al., 2003; Kriajevska et al., 1994; Watanabe et al., 1993). However, there are no clear *Dictyostelium* homologs of supervillin or Mts1 recognizable in the completed genome database. Thus, it remains to be determined whether *Dictyostelium* contains proteins that connect actin and myosin filaments.

Myosin II-regulating proteins

Apart from the myosin and MHCK mutants mentioned before, several *Dictyostelium* mutants have been described that are deficient in cAMP-induced myosin filament translocation. These include mutants that are defective in cGMP production or binding, mutants lacking the p21-activated kinase PAKa and mutants that lack the cAMP-specific phosphodiesterase RegA. It will be interesting to know how the cGMP pathway works together with PAKa, RegA and the MHCKs to regulate the localization and activity of myosin II. The signal transduction pathways regulating myosin II are discussed below and schematically outlined in Fig. 2.

Myosin II regulation by cGMP

One set of mutants that display altered myosin II localization is related to the signal transduction molecule cGMP. This molecule is rapidly produced after stimulation with chemoattractants and plays a role in *Dictyostelium* chemotaxis (reviewed in (Bosgraaf and Van Haastert, 2002)). Cyclic GMP is synthesized by two guanylyl cyclases, whereas its degradation is performed by three phosphodiesterases (Roelofs and Van Haastert, 2002; Kuwayama et al., 2001; Bosgraaf et al., 2002b). Furthermore, a high-affinity cGMP-binding protein (GbpC) has been identified, which is a complex protein containing RasGEF, small GTPase, leucine-rich repeat and kinase domains (Goldberg et al., 2002). Mutants that lack GbpC or the two guanylyl cyclases display reduced chemotaxis efficiency that is presumably caused by the complete loss of cAMP-induced myosin II translocation to the cortex (Bosgraaf et al., 2002a; Bosgraaf et al., 2005). Interestingly, these mutants also display a somewhat reduced MHC phosphorylation response upon cAMP stimulation (Bosgraaf et al., 2002a; our unpublished results). Since decreased heavy chain phosphorylation by itself should lead to increased myosin assembly (Egelhoff et al., 1993), the reduced MHC phosphorylation cannot account for the observed loss of cAMP-induced myosin translocation. In fact, the decreased heavy chain phosphorylation is possibly a consequence of the reduced association of myosin with the cortex, since the majority of MHC kinase activity resides in the Triton-insoluble fraction (Berlot et al., 1987). Thus, cGMP and GbpC induce myosin filament translocation to the cortex, presumably without directly altering the MHC phosphorylation state. Interestingly, the cGMP mutants display no aberrancies in cAMP-induced F-actin formation, suggesting that GbpC may act specifically on the myosin filament translocation event during chemotaxis (Bosgraaf et al., 2005).

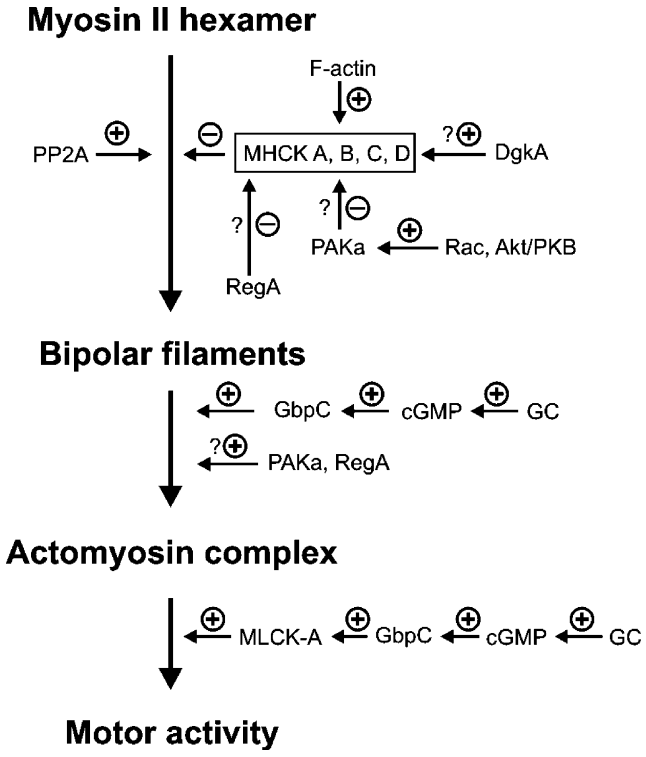

Fig. 2. Signal transduction pathways regulating myosin II. Myosin filament formation is regulated by the phosphorylation of three threonines in the heavy chain tail. Phosphorylation is performed by four myosin heavy chain kinases (MHCK A-D). The activity of the MHCKs is regulated by F-actin (MHCK A) and possibly also by the cAMP phosphodiesterase RegA, the p21-activated kinase PAKa, and the diacyl glycerol kinase DgkA. PAKa is in turn regulated by Rac and Akt/PKB. Dephosphorylation by the heavy chain phosphatase PP2A induces the formation of myosin filaments which can complex with filamentous actin to form actomyosin. During chemotaxis, this process is regulated by the second messenger cGMP that is produced by two guanylyl cyclases (GC) and can bind to GbpC. Furthermore, myosin filament translocation may also be regulated by RegA and PAKa. Although actomyosin can exert a number of actions in the cell, some functions require myosin II motor activity. Motor activity is regulated by the phosphorylation of serine 13 of the regulatory light chain, which is performed by MLCK-A, which is directly or indirectly phosphorylated by GbpC.

Myosin II regulation by PAKa

Another protein that is involved in myosin localization is the p21-activated kinase PAKa. It is composed of an N-terminal domain that is required and sufficient for its localization, a Rac1-interacting CRIB domain, and a serine/threonine protein kinase domain. Null mutants had no apparent phenotype in one study, but displayed a number of anomalies in a different genetic background (Chung and Firtel, 1999; Müller-Taubenberger et al., 2002). This included a mild cytokinesis defect when grown in suspension, delayed aggregation,

the formation of multiple F-actin-filled pseudopodia and decreased chemotaxis efficiency. The latter defect is caused by the inability to suppress lateral pseudopodia and by inefficient uropod retraction. Similar defects were also seen in cells overexpressing a kinase-dead mutant of PAKa, indicating that the kinase domain is essential for its function. Since these defects are reminiscent of mutants with blocked myosin function, it is conceivable that PAKa acts via myosin II. Indeed, myosin-GFP, which resides in the posterior of moving wild-type cells, was much more diffusely localized in *paka*$^-$ cells. Moreover, myosin did not translocate to the detergent-insoluble fraction upon cAMP stimulation, although basal levels were only slightly altered. Interestingly, PAKa localizes to the posterior of translocating cells, similar to myosin II filaments (Chung and Firtel, 1999; Müller-Taubenberger et al., 2002). These data suggest that PAKa is required for the localization of myosin II. In a subsequent study it was shown that Akt/PKB phosphorylates PAKa on a threonine residue that is located in the N-terminal localization domain (Chung et al., 2001). The phosphorylation state of this residue plays a role in the localization and activity of PAKa. This reveals an interesting link between the PI3K-activated PIP3 pathway that activates Akt/PKB and PAKa that influences myosin II localization. Although PAKa cannot directly phosphorylate MHC, it could exert its action by inhibiting one or more MHCKs or by activating a MHC phosphatase (Chung and Firtel, 1999). Alternatively, PAKa may act on myosin by altering the F-actin cytoskeleton, which is also altered in *paka* mutants.

Myosin II regulation by RegA

Myosin localization is also altered in a targeted disruption mutant of the gene encoding the cAMP-specific phosphodiesterase RegA. This enzyme acts as a response regulator and is activated upon aspartate phosphorylation by the histidine phosphotransfer protein RdeA (Thomason et al., 1999). Moreover, the inhibition of RegA by the cAMP-induced activation of ERK2 is thought to play a role in the generation of pulsatile cAMP production (Maeda et al., 2004). Cells lacking RegA display elevated cAMP levels and consequently develop unusually rapid (Kim et al., 1998; Thomason et al., 1998). Furthermore, these mutants display aberrant chemotaxis due to the inability to suppress lateral pseudopodia (Wessels et al., 2000). This is presumably caused by a diminished myosin II localization to the cortex in the front of a cAMP wave. It is unknown whether this defect is accompanied by altered MHC phosphorylation levels. The only known cAMP target in *Dictyostelium* is protein kinase A (PKA). Upon cAMP binding, the regulatory subunit of PKA dissociates from the catalytic subunit which is thereby activated (reviewed in (Veron et al., 1988)). A mutant lacking the regulatory subunit of PKA displays constitutive PKA activity and is expected to have similar defects as *regA* null mutants. However, although mutants lacking the regulatory PKA subunit indeed display the same inability to suppress lateral pseudopod formation, the myosin II localization is unaltered in these cells (Zhang et al., 2003). The difference may be explained by the elevated, but still pulsatile PKA activation that occurs in *regA*$^-$ cells compared to the continuously increased PKA activity in the mutant lacking the regulatory subunit of PKA. Alternatively, RegA may have other functions independent of its phosphodiesterase activity.

The regulation of myosin motor function by regulatory light chain phosphorylation

In *Dictyostelium*, myosin II motor activity is regulated by the phosphorylation of RLC serine 13. Stimulation with cAMP induces a transient increase in serine 13 phosphorylation, leading to a four- to six-fold increase in myosin motor activity (Griffith et al., 1987; Liu et al., 1998; Uyeda et al., 1996). *Dictyostelium* RLC in which serine 13 is mutated to a non-phosphorylatable alanine is no longer phosphorylated in vitro or in vivo, suggesting this is the only residue that becomes phosphorylated (Ostrow et al., 1994). Remarkably, this RLC S13A mutant can rescue the *rlc* minus phenotypes, which demonstrates that phosphorylation of RLC is not required for myosin function. However, S13A mutants do have a mild chemotaxis defect resulting from suppressed lateral pseudopod formation and the inability to depolarize at the peak of a cAMP wave (Zhang et al., 2002). An unconventional myosin light chain kinase (MLCK-A) has been identified in *Dictyostelium,* which – in contrast to mammalian MLCK – is not regulated by calcium (Tan and Spudich, 1990, 1991). Instead, it is regulated by autophosphorylation on threonine 289 and by phosphorylation on threonine 166 by an upstream kinase (Smith et al., 1996; Tan and Spudich, 1990; Tokumitsu et al., 2004). Furthermore, cGMP can enhance the activity of MLCK-A in fresh lysates, although cGMP cannot directly activate MLCK-A (Silveira et al., 1998). This is in agreement with the finding that mutants lacking cGMP or the GbpC show greatly reduced RLC phosphorylation upon cAMP stimulation (Bosgraaf et al., 2002a; L. Bosgraaf., P. van Haastert and J.L. Smith, unpublished observations). Most likely, cGMP activates GbpC, which phosphorylates MLCK-A either directly or via an intermediate kinase. In agreement with this, cAMP-induced MLCK-A phosphorylation is markedly reduced

in mutants lacking GbpC (L. Bosgraaf, J.L. Smith, and P. van Haastert, unpublished observation). Thus, the motor activity of myosin II is regulated via a protein kinase cascade that is activated by cGMP.

Conclusions

It has proven useful to discern a structural and a motor mode for *Dictyostelium* myosin II. The structural action results from the local association of myosin filaments to the actin cytoskeleton that leads to increased cortical tension and the suppression of lateral pseudopodia. The motor activity is only required for cytokinesis in suspension and active retraction of the uropod. It is important to note that although the structural mode of myosin can function independently of the motor activity in certain mutants, this separation will not likely occur in wild-type amoebae. Undoubtedly, the processes responsible for filament assembly, filament translocation and motor regulation are delicately interrelated both temporally and spatially. This is supported by the finding that myosin association to the cortex and myosin motor activity are both regulated by the second messenger cGMP. Furthermore, phosphorylation of the RLC has been shown only to have an effect on motor activity if myosin resides in its filamentous state (Liu et al., 1998). More studies are required to elucidate the pathways regulating myosin action and the presumably complex interplay between them.

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